added by these amendments. Reconsideration is respectfully requested in light of these amendments and the following remarks.

## I. Finality of Restriction Requirement

The Examiner has made final the Restriction Requirement of Group I, claims 1-28 and Group II, claims 29-52. Accordingly, in an earnest effort to advance the prosecution of this case, Applicants have canceled claims 29-52, without prejudice. However, in light of the finality of this Restriction Requirement, Applicants reserve the right to file a divisional application to the canceled subject matter.

## II. Rejection of Claims 1-4 and 6-28 under 35 U.S.C. § 102(b)

Claims 1-4, 6-24 and 28 have been rejected under 35 U.S.C. § 102(b) as being anticipated by Wicks et al. (WO 94/27156). The Examiner suggests that Wicks et al. disclose use of antibodies and detectable labels and markers to detect troponin I and troponin C in a complex sandwich assay having immobilized solid phases for the purpose of assaying irreversible cardiac damage from biological samples such as blood. Further, the Examiner suggests that Wicks et al. teaches and claims use of antibodies to specific fragments of troponin I.

Claims 1-2, 8-21 and 25-28 have also been rejected under 35 U.S.C. § 102(b) as being anticipated by Takahashi et al. (WO 96/10078). The Examiner suggests that Takahashi et al. disclose the use of antibodies to detect myosin light chain 1 in a complex sandwich assay having immobilized solid phases for the purpose of assaying irreversible cardiac damage from biological samples such as blood.

Applicants respectfully traverse these rejections.

At the outset, it is respectfully pointed out that the claims of the instant application are drawn to methods for assessing muscle damage in a subject by evaluating for the presence or absence of a **myofilament protein modification product**. Myofilament protein modification products are defined in the specification at page 10, line 21, through page 11, line 15, and are inclusive of modified forms of myofilament proteins or peptide fragments of myofilament proteins such as α-actinin, a troponin, or myosin light chain 1. Exemplary myofilament protein modification products include peptide fragments of α-actinin, a carboxyl-terminal region of troponin I, an amino-terminal region of troponin I, peptide fragments of troponin T, peptide fragments of myosin light chain 1 and covalent complexes of two intact proteins or protein fragments.

In contrast, the teachings of Wicks et al. are focused upon

methods for distinguishing normal cardiac troponin I from normal skeletal troponin I. There is no teaching nor suggestion of detecting modification products, i.e. modified forms of troponin I, peptide fragments thereof, or covalent complexes of proteins or protein fragments of troponin I, for assessing muscle damage. While Wicks teaches a peptide fragment and preferred segments of this fragment to which antibodies specific against cardiac troponin I can be raised (see page 5, lines 5-17), Wicks does not teach detection of this or any other troponin peptide fragment in a biological sample obtained from a subject nor a method for assessing muscle damage based upon detection of such a fragment.

Similarly, the teachings of Takahashi et al. are related to detection of normal myosin 1 light chain, not modification products thereof.

MPEP § 2131 is quite clear; to anticipate a claim, the reference must teach every element of the claims. Since neither Wick et al. nor Takahashi et al. contain teachings of a method for assessing muscle damage by evaluating for the presence or absence of a myofilament protein modification product, these references cannot anticipate the pending claims. Withdrawal of these rejections is therefore respectfully requested.

In an earnest effort to clearly highlight distinguishing factors of the present invention from prior art teachings such as Wicks et al. and Takahashi et al., Applicants have added new claims 53-55 which are drawn to specific myofilament protein modification products, levels of which can be assessed to determine muscle damage. Support for these claims can be found in the specification at page 10, line 21, through page 11, line 15. No new matter is added by these claims.

**III. Objection to Claim 5**

Claim 5 has been objected to as depending from a rejected claim. Although Applicants believe that the above remarks overcome the pending rejections of the independent claim from which claim 5 depends, in an earnest effort to be completely responsive, Applicants have amended claim 5 to be independent. Withdrawal of this objection is therefore respectfully requested.

**IV. Amendments to Specification**

The specification has been amended to correct inadvertent typographical errors, namely in the Figure Descriptions, references to Figures in the specification, and characterization of data

presented in the Figures, which were noted during preparation of this response. No new matter has been added by these amendments and entry of these amendments is respectfully requested.

**V. Correction to Figure 4**

Figure 4 has been corrected in accordance with the Figure Description at page 6, line 15, of the specification, to include an arrow to the modification product depicted in Panel G. No new matter has been added by this correction. Entry of this corrected Figure is therefore respectfully requested.

**VI. Amendment to Claim 17**

Claim 17 has been amended to include fatigue as a condition from which the muscle damage is due. Support for this amendment can be found in the specification at page 35, lines 25-29, page 36, line 28, through page 37, line 1, and Figure 11. No new matter has been added by this amendment and entry is respectfully requested.

**VII. Conclusion**

Applicants believe that the foregoing comprises a full and complete response to the Office Action of record. Accordingly,

favorable reconsideration and subsequent allowance of the pending claims is earnestly solicited.

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

**In the Specification:**

The paragraph, beginning on line 20, of page 4 has been amended as follows:

Figure 1 is a graph showing the effect of reperfusion on the isometric force/pCa relation of Triton X-100 skinned cardiac muscle fiber bundles obtained from isolated rat hearts that experienced increasing durations of ischemia. Isometric force was measured as a function of increasing calcium concentrations for each skinned trabecula muscle bundle obtained from rats which had undergone 15 min of equilibrium followed by: 45 min of perfusion (control n=4,○), 15 min ischemia (n=4,△), 15 min ischemia followed by 45 min of reperfusion (n=4,▽), 60 min of ischemia (n=4,□) or 60 min ischemia followed by 45 min reperfusion (n=6,◇). Force is plotted as the percent of either the maximum force produced by the control skinned muscle fiber bundles (Fmax=force of fiber/maximum calcium-dependent force of control fiber bundle (100%), panel A) or as the relative force (maximum calcium-dependent force of the fiber bundle = 100%, panel B) with respect to changing calcium concentration. The experimental protocols of the Langendorf perfusion and skinned muscle fiber bundle analysis are described

below. Data are presented as ±SEM for skinned fibers obtained from 4 to 6 rat hearts for each experimental condition. Where the error bars are not shown, the standard error is small and lies within the symbol.

The paragraph, beginning on line 5, of page 6 has been amended as follows:

Figure 4 shows the results of an SDS-PAGE analysis of skinned left ventricle tissue samples from isolated rat hearts. Tissue samples obtained from hearts which experienced 15 min equilibration followed by either 45 min perfusion (control, 1), 15 min ischemia followed by 45 min reperfusion (i.e., 15/45; 2), 60 min ischemia (3) or 60 min ischemia followed by 45 minutes reperfusion (i.e., 60/45; 4) were skinned in 50% glycerol prior to being prepared for SDS-PAGE analysis. Panel A shows the coomassie blue stain of the 12.5% crosslinked gel. Panels B-F show corresponding western blots using anti-α-actinin (panel B), anti-TnI peptide residues 136 to 148 (MAb E2, panel C), anti-TnT (panel D), anti-TM (panel E), and anti-MLC1 (panel F) antibodies. Panel G shows the western blot of a 10% SDS-PAGE of control tissue and tissue obtained from rats which experienced 60 min ischemia ~~(2)~~ (3). The western blot was

probed with anti-α-actinin antibody. Modification products are indicated by arrows.

The paragraph, beginning at line 1, of page 7 has been amended as follows:

Figure 6 shows the results of an SDS-PAGE analysis of isolated myofibrils from control and globally ischemic rat hearts. Left ventricular tissue samples obtained from isolated rat hearts were placed in saline in plastic bag for 60 min at either 4°C (control, 1) or 39°C (global ischemia, 2). Panel A shows the coomassie blue stain of the 12.5% crosslinked gel. Panels B to F show corresponding western blots using anti-α-actinin (panel B), anti-TnI peptide residues 136 to 148 (panel C), anti-TnT (panel D), and anti-MLC1 (panel E) antibodies. Modification products are indicated by arrows. The data reveal a loss of α-actinin in the global ischemic myofibrils <u>and degradation of TnI and MLC1, respectively.</u>

The paragraph, beginning on line 10, of page 30, has been amended as follows:

Results of the SDS-PAGE analysis and subsequent western blots of rat heart reperfusion effluent are shown in Figure 3, and of

tissue from global ischemic rat hearts in Figure 6, wherein MLC1 modification product is identified by an arrow (Figure 6E). ~~Figure 3D shows a that a 32kDa covalent~~ Figure 8 and Figure 10C and 10D show that complexes ~~is~~ are formed from fragments of TnI, TnT, and TnC. Figure 4 shows the SDS-PAGE analysis and subsequent western blots of rat skinned ventricular tissue, wherein TnI modification products can be seen (Figure 4C, arrow). Note that α-actinin was lost (Figure 4B) with mild ischemia, and α-actinin degradation (Figure 4G) appeared with more severe ischemia.

The paragraph, beginning at line 23, of page 31, has been amended as follows:

To identify the site of modification in troponin I, specific antibodies to the amino- and carboxyl-termini of troponin I were used to find out which antibodies bind to the different modification products. The antibodies MAb 10F2 (recognizes residues 188 to 199) and MAb 3350 (2F6.6) (recognizes residues 28 to 54) were used (Van Eyk et al. 1998, *Circ. Res.* 82:261-71). The various modification products were run on either a 12% SDS-PAGE or 10% T-PAGE (described in Schagger et al. 1987, *Analytical Biochemistry*, 166:368-79). The proteins were transferred to nitrocellulose using a 10 mM CAPS buffer pH 11.0 for 16 h at 27 V

(described in Towbin et al. 1979, *PNAS* 76:4350-54). The carboxyl-terminus is usually the first to be clipped (Figure 7), yielding residues 1 to 193 (Figure 9), but in addition there are further modifications occurring at the amino-terminus with more severe ischemia (~~Figure 9~~ Figure 8A). Further TnI degradation products were identified as listed in Table 4.

The paragraph, beginning at line 13, of page 38, has been amended as follows:

All tissue samples were excised and quickly washed in cold (4°C) saline before being frozen in liquid $N_2$ and stored at -70°C until prepared for SDS-PAGE analysis. Frozen tissue samples were homogenized in 25 mM Tris, pH 7.5, plus a cocktail of protease inhibitors (50 M phenylmethylsulfonyl fluoride, 3.6 M leupeptin, 2.1 M pepstatin A, and 10 mM EDTA). The protein contents of the homogenates were determined using Lowry assay. Protein samples were then prepared in Laemmli buffer (1% (w/v) SDS, 2.5 mM Tris, pH 6.5, 10% (w/v) sucrose, 0.025% (w/v) bromophenol blue) and ~~42~~ 1 mM dithiothreitol at a total protein concentration of 1 mg/ml before being stored at -20°C for later SDS-PAGE and western immunoblot analysis.

The comments following Table 3 have been amended as follows:

* The ischemia/reperfusion-induced modified TnI products observed in urea T-PAGE separated left ventricular tissue which underwent either 0/45, 15/45/ or 60/45 (~~Figure 9~~ Figure 8) were quantified from 8I-7 MAb Western blots (~~Figure 9A~~ Figure 8A). The quantity of each TnI component was determined as a percentage of the total TnI (intact and modified) present in each tissue sample. Only those products which are positively identified in Table 4 are included here, identified by their apparent molecular weight (~~Figure 9A~~ Figure 8A).

† The ischemia/reperfusion-induced modified TnI products observed from 8I-7 MAb affinity chromatography of 60/45 left ventricular tissue (~~Figure 11~~ Figure 10) were quantified from 8I-7MAb Western blots (~~Figure 11B~~ Figure 10B), and the amount of each TnI component determined as a percentage of the total in each sample.

‡ Control tissue, which experienced no ischemic episode, but 45 minutes of reperfusion.

§ The quantity of the two TnI-containing covalent complexes combined.

// Quantities less than 2% of total TnI could not be accurately determined.

The comments following Table 4 have been amended as follows:

* TnI products identified by their apparent molecular weights (~~Figure 9A~~ Figure 8A).

† Immunological analysis (Western blots, ~~Figures 9A, 11C~~ Figures 8A, 10C) of protein products bound to Mabs: strong (+), weak ($\pm$), or no binding (-).

‡ Electrophoretic mobility in alkaline urea PAGE (~~Figure 11~~ Figure 10): mobile (+, TnC containing), non-mobile (-, not containing TnC).

§ The amino acid sequences(s) of proteins which are the theoretical best match to the observed masses.

// Best match to the observed masses was determined by calculating the mass of rcTnI, rcTnT, and mouse cTnC, sequentially clipped from the N- and C-termini using the PeptideMass tool from the Swiss Institute for Bioinformatics website.

¶ The source of the TnI products indicates the peak from RP-HPLC analyzed 8I-7 affinity column fractions of 60/45 tissue (Fig 4).

# Mass determined by electrospray mass spectrometry.

** Mass determined by matrix assisted laser desorption/ionization mass spectrometry.

†† The difference between the observed and theoretrical masses is equal to that of a sodium ion (MW 35 Da), which is commonly found

associated with mass spectrometrically analyzed proteins (as a result of the ionization process).

**In the claims:**

Claims 29-52 have been canceled.

Claims 5 and 17 have been amended as follows:

5. (amended) ~~The method of claim 3~~ A method for assessing muscle damage in a subject, comprising evaluating for the presence or absence of at least two different myofilament protein modification products in a biological sample, wherein said at least two different myofilament protein modification products are from the same protein.

17. (amended) The method of claim 16, wherein the muscle damage is due to at least one condition selected from the group consisting of hypoxia, hypoxemia, ischemia, fatigue, and reperfusion.